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Cell Division: Eg'ing on Microtubule Flux

Dispatch

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Mitotic microtubules are moved toward spindle poles in a process known as flux. Several proteins responsible for flux have recently been identified, significantly advancing our understanding of chromosome movements in mitosis.

The process of division allows cells to reproduce, constantly generating new cells to replace those that wear out. During the mitosis stage of division, replicated chromosomes are separated by the mitotic spindle, a complex array of dynamic microtubule polymers, motor proteins and scaffolds [1]. Our understanding of how this dynamic and transient organelle assembles and moves chromosomes is advancing rapidly. Several recent papers [2–5], one in a very recent issue of *Current Biology* [5], report major steps forward in defining the molecular components responsible for driving spindle microtubule flux, a key component of chromosome movement.

The mitotic spindle is organized in a bipolar shape that ultimately segregates the replicated chromosomes to opposite poles (Figure 1). Microtubule polymers, built from tubulin subunits, form the major structural elements of the spindle (reviewed in [1,6]). The minus ends of these microtubules are bundled together at the spindle pole by several proteins, including NuMA and dynein, a minus end-directed motor. The opposite, plus ends are attached to the kinetochores, protein complexes that assemble on the centromere of each chromosome (Figure 1). Microtubules attached to kinetochores and spindle poles are not static: rather, the entire microtubule lattice moves from kinetochore to pole, even during metaphase, when the chromosomes are aligned at the spindle equator and the spindle length is held constant [7,8]. This microtubule movement is known as flux and is an important source of anaphase chromosome movement [9]. Flux also generates tension on the kinetochores, which regulates the activities of motor proteins localized to these kinetochores [10,11].

Flux requires that microtubules polymerize at the kinetochore, at their plus end, and depolymerize at the pole, at their minus end, and that the microtubules in their entirety move toward the pole (reviewed in [1,6]). What drives this flux within the spindle? Several possible molecular models are diagrammed in Figure 1. Microtubule assembly and disassembly reactions are theoretically sufficient to generate flux if tubulins add to microtubules at the kinetochore (plus end) and are lost from the pole (minus end). Preferential assembly at one end of a polymer and depolymerization at the other is termed treadmilling (model 1). In this model, treadmilling would be powered by hydrolysis of tubulin-bound GTP as the tubulin subunits assemble.

While such treadmilling is possible, it is more likely that motor proteins pull on the microtubules to generate flux. Two scenarios in which motor proteins drive flux are shown in Figure 1 (models 2 and 3). Each model incorporates a KinI protein, a type of kinesin that is not thought to have motility, but instead to use ATP hydrolysis to destabilize microtubule ends, causing them to depolymerize. It is possible to have a pole-bound KinI molecule that both pulls on the microtubule and destabilizes the microtubule minus end, thereby both reeling the microtubule into the pole and chewing up its minus end — a 'self-feeder'. Alternatively, two kinesins could work together: a plus end-directed kinesin could pull on a microtubule, reeling it into a pole-localized KinI that chews it up. Several recent studies have demonstrated that model 3 is the correct explanation for flux and have identified the proteins responsible.

The recent progress has come from the application of a convenient technique for detecting flux in two model systems for studying mitosis. To monitor flux, the microtubule lattice is marked by introducing a small amount of fluorescently tagged tubulin into the spindle [12]. Random addition of marked and unmarked tubulin molecules gives the lattice a speckled fluorescent pattern. Poleward flux is detected by movement of the speckles [12]. Fluorescent speckle microscopy has been used to study spindle flux in *Drosophila* embryos [3] and *Xenopus* egg extracts [2,5]. In *Drosophila* embryos, spindles assemble synchronously in a shared syncytial cytoplasm, making it easy to study a large number of spindles simultaneously. Spindles assembled in *Xenopus* egg extracts are frequently used to study mitosis because this *in vitro* system allows easy manipulation of component parts, for example by addition of antibodies or modified proteins to disrupt protein function or immunodepletion to remove specific proteins.

Two new studies [2,5] used *Xenopus* egg extract spindles to determine how flux is generated. Models 1 and 2 both predict that minus end depolymerization is coupled to microtubule translocation and that it would be impossible to have one without the other. In contrast, model 3 predicts that minus end depolymerization and microtubule translocation occur by separate mechanisms; if minus end depolymerization is blocked, spindles will get longer, and they will do so at the same rate as flux. This latter prediction has now been demonstrated by identifying conditions that disrupt minus end depolymerization. Gaetz and Kapoor [2] found that inhibition of either NuMA or the dynein/dynactin complex caused spindle microtubules to grow longer without disrupting poleward flux. Similarly, Shirasu-Hiza *et al.* [5]

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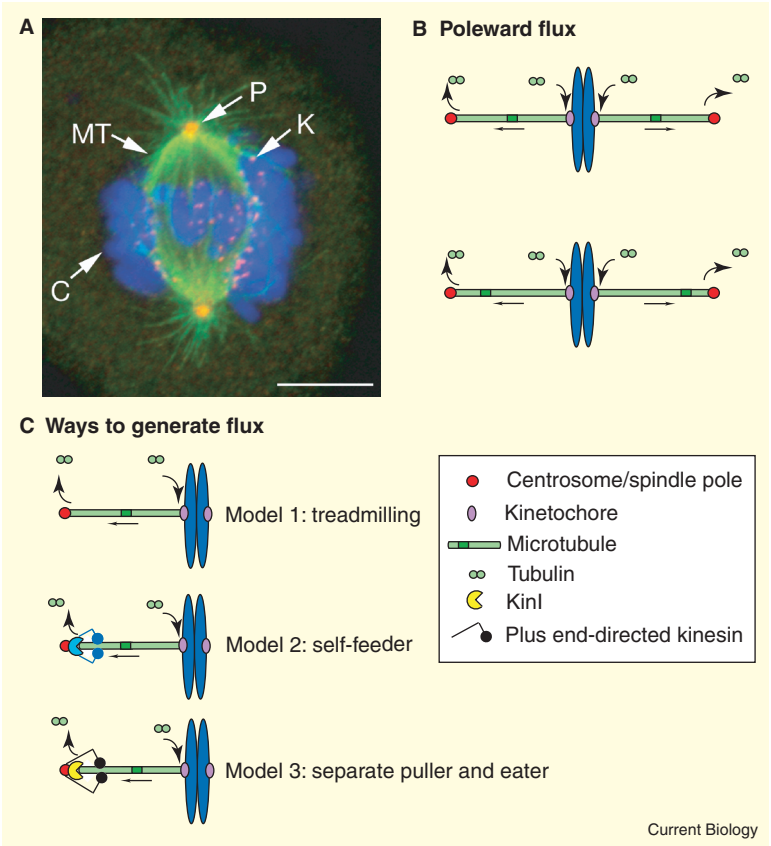

Figure 1. Mitotic spindle structure and microtubule flux.

(A) Immunofluorescent micrograph of a mitotic spindle in a human A549 cell. Microtubules (green, MT) originate at each of two centrosomes/spindle poles (red, P) and are organized in a bipolar shape. The plus ends of microtubules attach to kinetochores (purple, K) on each chromosome pair (blue, C). This cell is in prometaphase and chromosomes have not yet aligned at the spindle equator. Microtubules in the spindle are dynamic, constantly assembling and disassembling from their plus ends [6]. At the same time, the entire microtubule lattice is undergoing flux — moving toward the spindle pole [1,6]. Scale bar is 10 μm. (B) Schematic diagram of flux. Spindle components are diagrammed using the same colors as in A. Tubulin subunits add to the plus ends of microtubules attached to kinetochores and exit at the spindle poles. By marking a region of the microtubule lattice (shown in darker green), it is possible to detect movement (flux) of the microtubule toward the pole. This movement is shown schematically by the poleward movement of the darker green segments. (C) Molecular models of flux generation. In model 1, microtubule treadmilling — the addition of subunits at one end and subtraction at the other — is sufficient to generate flux. In model 2, microtubule depolymerization at the pole and poleward movement of the microtubule are both driven by a KinI protein anchored at the pole. In model 3, a plus end-directed kinesin pulls the microtubule toward the pole and a separate KinI depolymerizes the microtubule's minus end. All models require addition of tubulin subunits at microtubule plus ends. Proteins responsible for regulating assembly at this end are not considered here (see [1,6] for more information).

found that disrupting dynein/dynactin and simultaneously adding a non-motor tail fragment of XKLP2, a plus end-directed kinesin, caused spindles to elongate, again without disrupting flux. It is not yet known how the XKLP2 tail fragment, in combination with the excess p50 protein used to disrupt the dynactin complex, inhibited minus end depolymerization, but both reagents were necessary [2]. Importantly, both groups [2,5] found that their inhibitory reagents caused spindles to grow longer at the same rate as microtubules moved toward the pole, indicating that microtubule translocation had been uncoupled from minus end depolymerization. Together, these data indicate that depolymerization is not required to generate microtubule translocation, eliminating models 1 and 2; instead, the data indicate that separate proteins move the microtubule lattice and chew it up, as in model 3.

The KinI responsible for stabilizing microtubule minus ends has been identified in *Xenopus* extracts as Kif2a [2]. The localization of Kif2a to spindle poles requires the dynein/dynactin complex and NuMA [2], indicating that disruption of dynein, NuMA and perhaps XKLp2 cause spindle elongation by removing Kif2a from the poles.

Recent experiments using *Drosophila* embryos also support an important role for KinI proteins in determining spindle size and regulating microtubule stability. Rogers *et al.* [3] found that inhibition of the KinI protein KLP10a resulted in longer spindles about 60% of the time. Flux was also blocked in these embryonic spindles, suggesting that KLP10a may both destabilize microtubule minus ends and generate the force for flux (model 2) [3]. KLP10a inhibition also results in excess microtubule polymerization and causes monopolar spindle formation in 30% of spindles, suggesting that the function of KLP10a in determining spindle morphology may be more complex than in models 1, 2 or 3 (see below).

While it is not yet clear whether a KinI can generate flux in *Drosophila* embryos, a plus end motor able to generate flux has now been identified in *Xenopus* extract spindles as the tetrameric, bipolar kinesin Eg5 [5]. Identifying Eg5 as the flux motor was not as simple as just inhibiting the motor's activity, even though a highly selective small molecule inhibitor, monastrol, is available [13]. Inhibiting Eg5 results in monopolar spindles because the spindle poles collapse back toward each other [14], indicating a role for Eg5 in generating a bipolar spindle shape. Shirasu-Hiza *et al.* [5] were able study the role of Eg5 in bipolar spindles because they found that normal spindle shape was retained if Eg5 and dynein/dynactin were both inhibited. They then examined flux in bipolar spindles treated with a combination of inhibitors. Minus end depolymerization was blocked by adding excess p50 protein plus the tail domain of XKLP2, while monastrol

was used to inhibit Eg5. Under these conditions both flux and spindle elongation stopped, indicating that Eg5 is responsible for microtubule translocation toward the pole [5]. Taken together with results from Gaetz and Kapoor [2], spindle microtubule flux in Xenopus extract spindles requires Kif2a to chew up the minus end and Eg5 to pull the microtubule into the jaws of Kif2a.

The results from studies on *Xenopus* egg extracts are likely to be relevant to human somatic cells, even though the resultant change in spindle morphologies differ. Ganem and Compton [4] recently identified Kif2a as an important determinant of spindle structure in human cell lines. They find that siRNA-mediated depletion of Kif2a results in monopolar spindle formation, reminiscent of the 30% monopolar spindles formed in *Drosophila* embryos after inhibition of KLP10a [3]. From a series of experiments, Ganem and Compton [4] suggest that depletion of Kif2a reduces flux, which in turn reduces tension at the kinetochore. The reduced tension switches the kinetochore into a state in which microtubules depolymerize from their plus ends [11]; this depolymerization could pull the spindle poles toward each other, generating a monopolar spindle shape.

In the future it will be important to simultaneously track kinetochore movements and flux [11] to determine how changes to flux and microtubule assembly regulate tension at the kinetochore and chromosome movement. Importantly, we now know the relevant proteins at the spindle pole, providing new tools to investigate mitotic chromosome movements.

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